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A novel glycopeptide resistance operon in environmental *Rhodococcus equi*

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Background

Vancomycin and teicoplanin are last resort glycopeptide drugs for treatment of MRSA and enterococcal infections. They inhibit cell wall formation by binding to the D-Ala-D-Ala terminal residues of peptidoglycan precursors. Resistance is due to synthesis of low affinity precursors that terminate with either D-Ala-D-Lac or D-Ala-D-Ser (1). VanA-type is the most common glycopeptide resistance in enterococci and is characterized by inducible high-level resistance to both vancomycin and teicoplanin (2).

Rhodococcus equi is Gram-positive, soil coco-bacillus that causes severe bronchopneumonia in horses. It can also cause fatal infections in immunocompromised humans (3). Van^R has been reported in human clinical *R. equi* isolates (4), however the resistance mechanism is unknown.

OBJECTIVE: To elucidate the mechanism of glycopeptide resistance in a vancomycin resistant *Rhodococcus equi* isolated from Danish soil.

Methods

Expression of resistance. The Bioscreen^R was used to analyze the growth curves of *R. equi* RE-S7B non-exposed and exposed to 8 mg/l vancomycin (without prior exposure to vancomycin and with pre-exposure to vancomycin) over 2 days at 30 °C. OD₆₀₀ was measured every 30 min. Experiments were done in triplicate. In addition, minimum inhibitory concentration (MIC) was also tested by Etest® on RE-S7B pre-exposed to increasing concentrations of vancomycin.

Whole genome sequencing. RE-S7B was sequenced using Illumina paired-end (PE) technology (500bp library). *De novo* assembly was done using Geneious v6.3, *van*-like genes were annotated using CLC genomic work bench and NCBI BLAST.

Results and Conclusions

Resistance in RE-S7B is inducible

RE-S7B had a vancomycin MIC >32 µg/mL and teicoplanin MIC = 8 µg/mL. At sub inhibitory concentration of vancomycin, vancomycin exposed cells resume growth faster than non-exposed cells (FIG.1). The MIC increased proportionally when exposed to higher vancomycin concentrations (FIG.2).

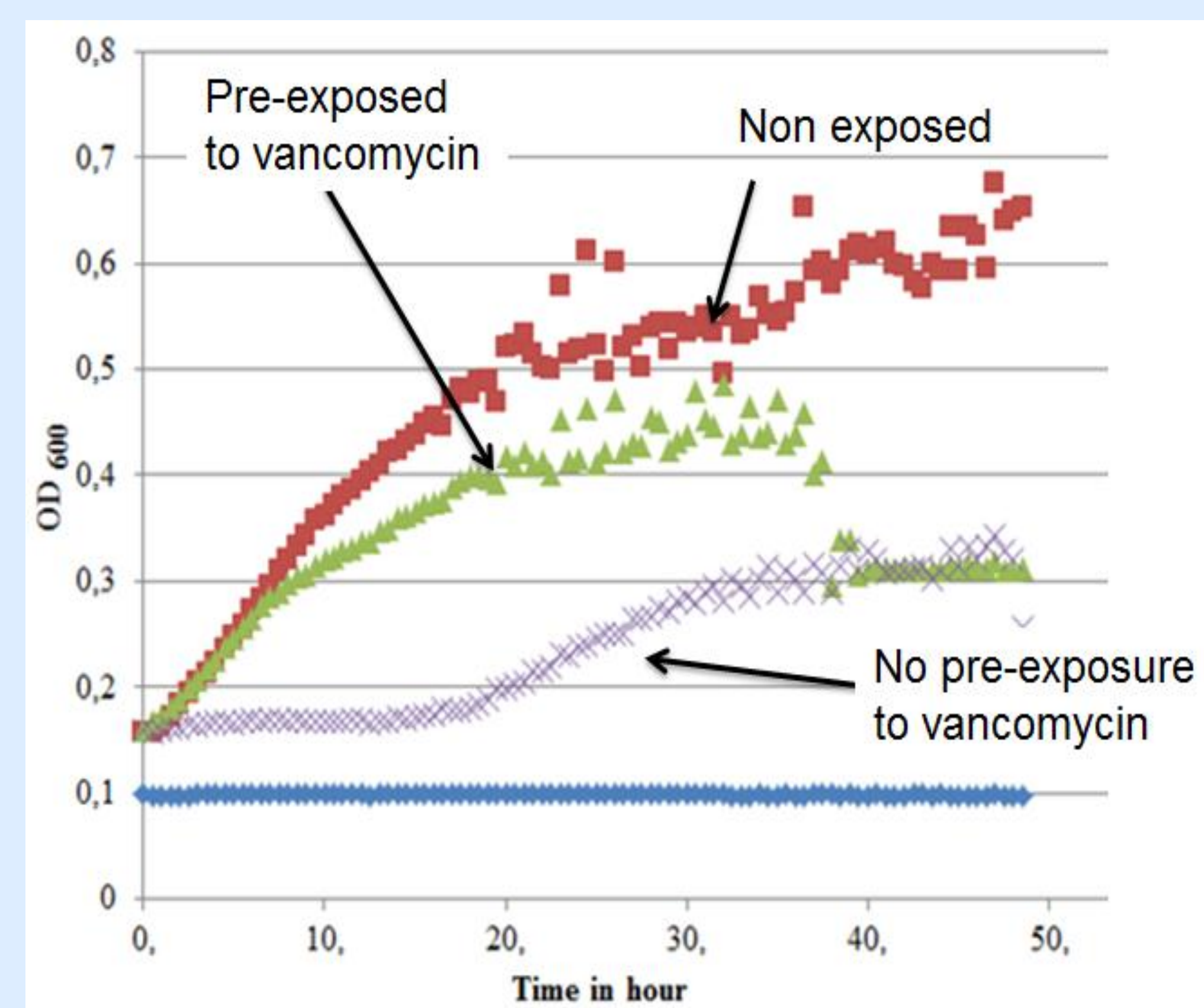


Figure 1: Glycopeptide resistance expression of RE-S7B.

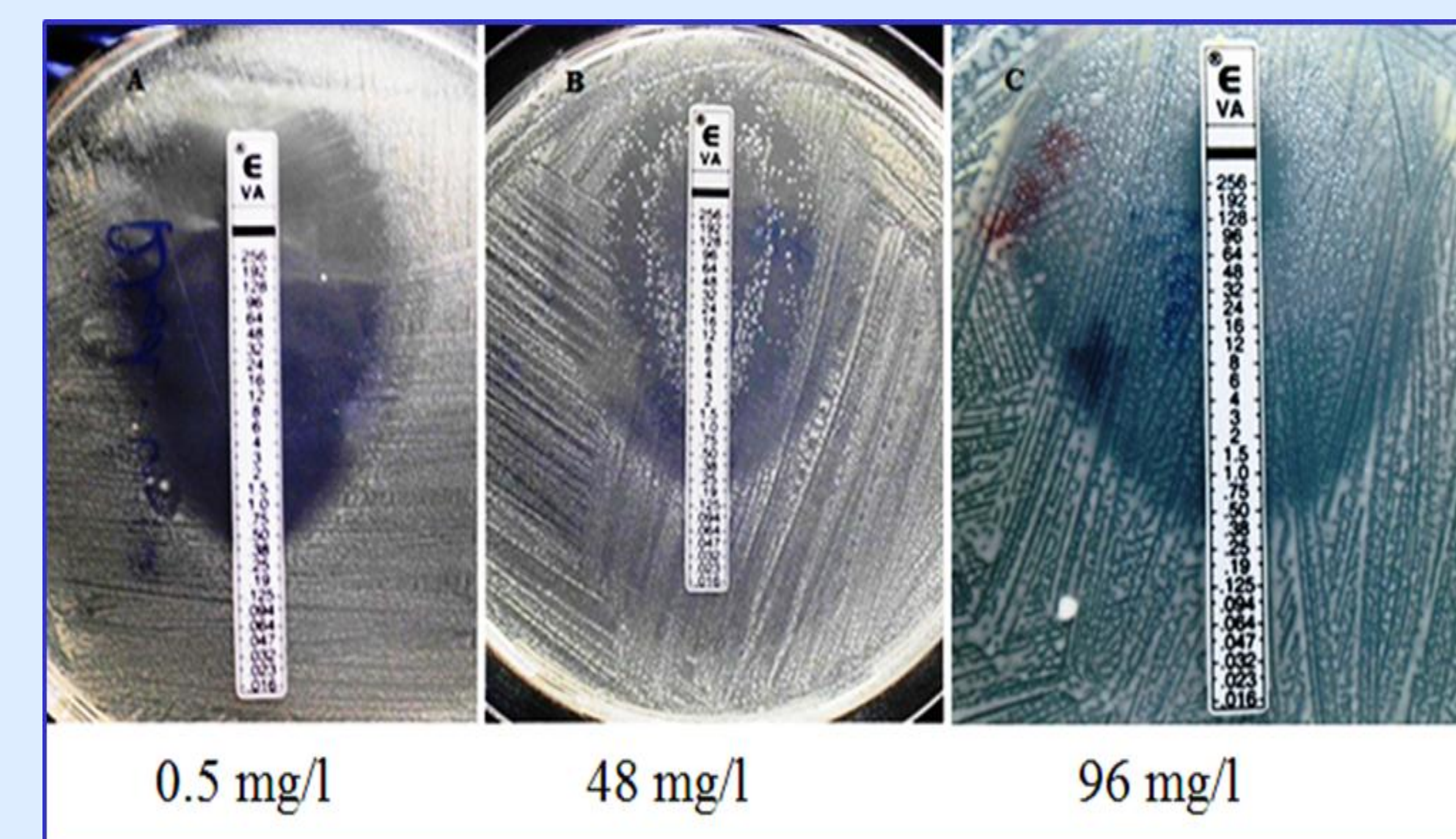


Figure 2: Vancomycin MIC values of RE-S7B recovered from BHI agar plates with no vancomycin (A), 8 mg/l of vancomycin (B) and 20 mg/l of vancomycin (C).

RE-S7B harbors a unique *van* operon

RE-S7B harbors a *vanA*-like operon consisting of a *vanHAX* cluster and a two-component regulatory system, displaying 60-63% and 39-41% amino acid identity to the enterococcal *vanA* genes, respectively (FIG. 3). The gene organization is unique and includes a novel transposase type and additional putative open reading frames. The proposed name for this novel operon is *van_{RE}*.

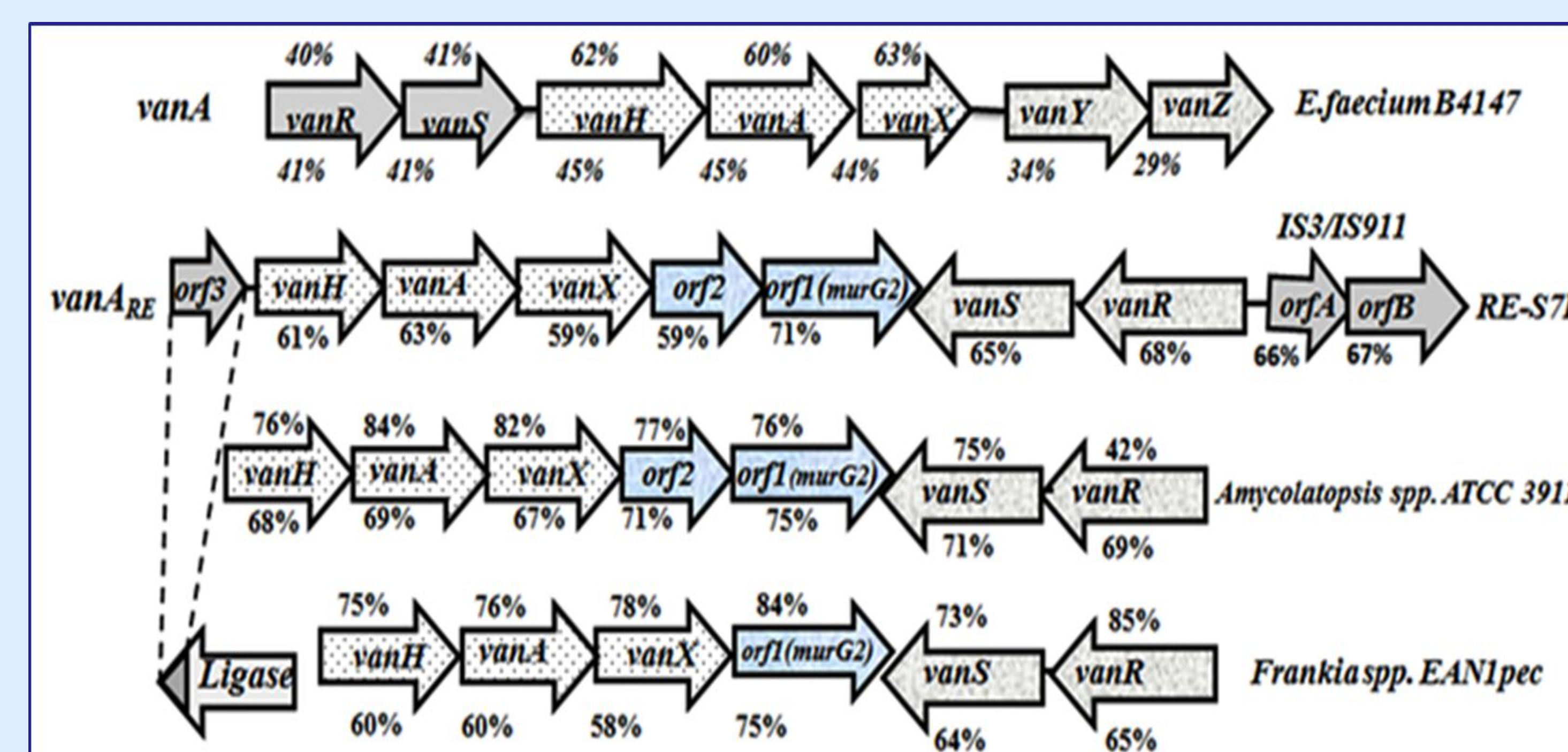


Figure 3: Organization of *vanA* in *E. faecium*, *van_{RE}* in RE-S7B, *van*-like clusters in *Amycolatopsis* spp. ATCC 39116 (NCBI BLAST) and *Frankia* spp. EAN1pec (NCBI BLAST). Arrows indicate direction of transcription. Percentages below and above the arrows indicate GC content and Nucleotide identity of the corresponding gene to *van_{RE}* operon in RE-S7B.

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